in a significantly increased the crosslinking of cysteines at the ICL4:NBD1 interface compared to the inhibition of phosphorylation with adenyl cyclase inhibitors. This suggests that phosphorylation modifies the ICL4 and NBD1 interface. These studies further our understanding of the molecular mechanisms underlying phosphorylation dependent gating of CFTR.

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Biphasic Influence of Bulk Anionic Phospholipids for PIP2 Gating of Kir2.1 Channels through Binding to Two Distinct Sites

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Inwardly rectifying potassium (Kir) channels regulate cell excitability and potassium homeostasis. Our recent analyses show that Kir2.1 channels have a distinct ('Secondary') anionic phospholipid (PL(-)) binding site, in addition to the crystallographically determined ('Primary') PIP2 activating site. Docking results suggest that PL(-)s can bind to either site and therefore might compete with PIP2 at the 'Primary' site and inhibit. To test this prediction we performed the following assays with purified human Kir2.1 channels reconstituted in liposomes. First, Kir2.1 activity was measured with a fixed PIP2 content and with increasing content of various PL(-)s. At higher PL(-) levels, inhibition was observed that correlated well with predicted affinity at the 'Primary' site. The 'Secondary' site is generated by residues K64 and K219. K64C mutant channels are insensitive to PL(-) and only weakly PIP2-activated, but high PIP2 sensitivity is regenerated by tethering of K64C to the membrane by decyl-MTS modification. Inhibition by PL(-)s was more potent in decyl modified 'Secondary' site single (K64C) and double (k64C/K219A) mutant channels. It's likely that PL(-) binding at the 'Primary' site is augmented in these mutants as a consequence of increased effective PL(-) in the membrane as well as reduced electrostatic repulsion from the PL(-) at the 'Secondary' site. Finally PIP2 sensitivity was measured in the presence of increasing PL(-)s. The apparent PIP2 Kd was left-shifted at low PL(-) (as expected for the activatory effect at the 'Secondary' site), but shifted back to the right at higher PL(-)s, consistent with an inhibitory effect of bulk PL(-) at the 'Primary' site, if present at high enough levels in the membrane. Such interplay between PIP2 and other PL(-)s on Kir2.1 channel gating can be predicted by a mechanistic two-site binding model.

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Conformational Changes that Opens TrkH Ion Channel

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A high intracellular potassium ion concentration is required for many essential cellular functions. To carry potassium ions across membranes, organisms must express potassium ion transport proteins, such as proteins in the Superfamily of Potassium Transporters (SKT). TrkH, a member of SKT, is required for bacterial growth in environments with low external potassium concentration. Previous studies showed that TrkH is an ion channel and ATP increases channel activity through an associated cytosolic protein, TrkA, which forms a homotetrameric ring. However, whether ATP regulation is preserved in TrkH of other organisms, and how ATP upregulates TrkH via TrkA are still not clear. Crystal structures of TrkH and TrkA suggest that movement of a tilted helix in TrkH and a conformational change in the TrkA tetrameric ring are required for the gating process. We have expressed and purified TrkH and TrkA from various pathogens, reconstituted them into liposomes and will examine the effects of ATP and other potential ligands on their activity. We will test our structure-inspired gating model by measuring the rate of crosslinking between strategically placed pairs of cysteine mutations.

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Identification of a Cholesterol Recognition/Interaction Amino Acid Consensus Domain in STIM1 and its Role in SOCE

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Store-operated calcium entry (SOCE) is a mechanism of calcium influx activated after the depletion of intracellular stores. The main components of this mechanism are Orai1, the calcium channel, and STIM1 a calcium sensor, which oligomerizes and activates Orai channels when calcium levels drop inside the endoplasmic reticulum. The activation of Orai1 requires a series of molecular rearrangements of STIM1, which culminate in the final exposition of a domain within STIM1 known as SOAR (Stim Orai Activating Region). Specialized plasma membrane regions enriched in sphingolipids and cholesterol, have been shown to modulate SOCE also. In this work, we identified in the SOAR region a cholesterol recognition/interaction amino acid consensus (CRAC) domain, which appears to be important for the STIM1-Orai1 interaction. Through mutagenesis of this domain, we found by FRET microscopy that SOAR and STIM1 mutated in the CRAC domain loose its capacity to interact with Orai1. This finding suggests that the association of STIM1 to cholesterol resides in a discrete region in STIM1 and may play an important role for the subsequent STIM1-Orai1 association.

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Modeling Structure of Human Papillomavirus Type 16 E5 Protein - a Molecular Dynamics Simulation Study

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Human papillomaviruses (HPV) infect mucosal and cutaneous epithelial cells leading to precancerous lesions. The HPV genome encodes three oncoproteins: E5, E6 and E7 from which E5 is the least understood. E5 of HPV-16, one of the "high risk" types of HPV strains, is an 83 amino acid hydrophobic membrane protein, with three hydrophobic transmembrane domains (TMDs). It oligomerizes into dimers or higher oligomers which form ion channels most likely by forming hexameric bundles. Computational modeling is used to obtain structural and functional features of this protein.
The three TMDs of E5 are identified using secondary structure prediction programs. The TMDs are assembled into a monomer by a 'Sequential' and 'Simultaneous' docking approach in which the conformational space of the three helices is screened by simultaneously altering distance, tilt and rotational angle between them. In a consequent step, loops linking the three helices are added using the program Loopy. Finally six monomers are assembled into a hexameric bundle. The bundle with TMD2 lining the pore remains intact allowing formation water filled pocket during entire 100 ns MD simulations. The water pocket formed by the six TMD2s of the bundle is mostly mantled by hydrophilic residues such as Ser-35, −37 and Thr-38, −40. Bundles with the other two TMDs, TMD1 and TMD3, mantling the pore are energetically almost undistiguishable from the bundle with TMD2 facing the pore.
With TMD2s facing the pore, ion channel activity possible. All asymmetric bundle architectures account for interactions of E5 with host proteins.

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Bcl-xL Destabilization of Ceramide Channels: Role of the Hydrophobic Groove

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Ceramide forms channels in the mitochondrial outer membrane capable of releasing proteins that trigger the execution phase of apoptosis. Bcl-xL inhibits the formation of these channels. Previous work indicated the hydrophobic groove of Bcl-xl may be the site that binds the ceramide channel resulting in its destabilization. Single residues in the hydrophobic groove were replaced with others with different biophysical properties generally resulting in a reduction of the potency of the mutated Bcl-xL but occasionally the potency was increased. Binding of fluorescent ceramide (C11 TopFlour ceramide) to the Bcl-xL protein was also affected by the mutations in a manner correlated to the functional changes in mitochondria. These results demonstrate that the hydrophobic groove is indeed the binding site. When Bcl-xL binds to fluorescent ceramide, the fluorescence is quenched compared to ceramide dissolved in isopropanol indicating that the binding only partially protects the fluorophore from quenching by water. The quenching is greater in the mutants indicating weaker binding and greater water contact. This view is supported by molecular dynamic simulations showing more motion of the bound ceramide in the mutant Bcl-xL and thus more access to water. This hydrophobic site also binds to the BH3 domain of Bax and inhibits Bax channel formation in the mitochondrial outer membrane. Some of mutants inhibited both channels to a similar extent. However, 2 mutants acted sufficiently differently on the two channels indicating overlapping but distinct binding sites. These mutants may be useful for distinguishing between these two modes of mitochondrial outer membrane permeabilization. (Supported by NSF grant MCB-1023008)

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Conformation Changes of a 7TM Receptor Caused by the Sample Environment as Studied by Multidisciplinary Biophysical Methods

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Choosing an appropriate sample environment is critical in structural biology, not only for trapping functionally relevant intermediate states of a membrane protein, but also for interrogation of structure, conformation and dynamics to elucidate structure-function relationships. Functional assays and structural studies should therefore be performed in the same environment.
Bacteriorhodopsin (bR), a member of the microbial rhodopsin family 7TM proteins, acting as a light-driven proton pump for light energy capture in